THE TOXIC EFFECT OF BEAUVERIA BASSIANA (BALS.) VUILL, ON INSECTS

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The post-mortem symptoms of insects killed by exposure to *Beauveria bassiana* (Bals.) Vuill. spores described by Dresner (1949) were in contrast to those heretofore ascribed to fungus invasion. This paper is an attempt to explain that variance; the paralysis of insects by entomogenous fungi is caused by a fungus-produced toxin. This toxin is produced by the spores during germination and by the hyphæ during growth. Under certain conditions this paralysis is followed by bacterial histolysis of the insect tissues. When bacterial histolysis occurs, the production of conidia is rare.

REVIEW OF THE LITERATURE

Experiments have been recorded in the literature relating to the toxicology and histology of fungus infection. Wallengren and Johansson (1929), Pilat (1938), Baldacci (1939), Toumanoff (1931), and Burnside (1930) mention the secretion of enzymes by the growing fungus. Wallengren and Johannson (1929) state that the toxic enzyme secreted by *Metarrhizium anisopliæ* (Metsch.) Sor. appears to produce nervous disorders in the insects ending in complete loss of activity; this stage is followed by death of the insect. Burnside (1930) found that an extract of the toxin produced by fungi (*Aspergillus* and *Mucor*) was a stomach poison. Wallengren and Johansson (1929) found that the time of penetration of the cuticle by the hypha was usually about five days; Sawyer (1933) includes observations with *Entomophthora sphærosperma* Fres. penetrating the cuticle in two to twelve hours after germination of the spores; paralysis took place a few hours later. Whinfield (1946) showed that germinating conidia of *Penicillium notatum* Westl. produced penicillin.

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Florey and Jennings (1942) reported that high concentrations of penicillin in the blood of humans caused inhibition or death of the leucocytes. Masera (1934) reported that *B. bassiana* inhibited the growth in culture of *Serratia marcesens* Bizio (*Bacillus prodigiosus* Flugge); within the insect the bacteria inhibited the growth of the fungus.

EXPERIMENTAL METHODS AND RESULTS

The accompanying tables describe the experiments and data obtained on tests of *B. bassiana* spores on housefly adults (*Musca domestica* L.), potato tuber worm larvæ (*Phthorimœa operculella* Zeller), and dock beetle adults (*Gastroidea cyanea* Melsh.). These tests indicate that the insect is paralyzed on exposure to the germinating spores in much less time than has been determined histologically for the penetration of the hypha into the body cavity. In many of the experiments described here, paralysis occurred in two hours or less after exposure of the insect to the germinating spores.

Most of the tests were repeated with a spore dust made from a new culture of spores. This new culture was obtained from the American Type Culture Collection, Washington, D. C. about two years after the first culture (1947). These tests were repeated primarily to insure that the toxic effect of the first spore dust was not due to an undetected contaminant. The series of tests with both spore dusts indicated similar results.

The fungus can produce this same paralytic effect after it has been killed; this is shown by experiments in which the potato tuber worm larvæ are treated with copper sulphate solution at the end of a two hour exposure to the germinating spores, treatment of the germinating spores with copper sulphate before the introduction of the insect, and finally, autoclaving of the germinated spores before introduction of the test insects. After these fungicidal treatments, no mycelia were observed to grow during a one week observation period. The rate of occurrence of paralysis and mortality did not differ substantially in all the tests involving fungicidal treatment compared with no treatment.

Experiments with housefly adults and potato tuber worm larvæ show that the toxin acts as a contact poison. The test in-

sects were ligatured at points as close as possible to the mouth. The results in these tests were comparable to those tests which did not include ligaturing of the insects.

The toxin can act through the alimentary tract; this was shown by the 100 percent mortality of housefly adults fed on milk which had been used as the medium for the culture of the fungus. In contrast, adult houseflies, fed spores which had not germinated, were not killed.

In these tests it was found that the germinating spores produce a toxin which is capable of causing paralysis of the insect. This paralysis is always evident in cases of fungus infection; with an invasion the spore germinates, the hypha penetrates the cuticle, and the toxin is produced in quantity within the host body. External sporulation usually follows. In the experiments reported here a very large number of spores was permitted to germinate on a filter paper, the toxin was produced in relatively great quantities, and the toxin was absorbed through the cuticle and acted as a contact poison.

Examination of the tuber worm larvæ a few hours after paralysis showed the body cavity to be filled with bacteria. This bacterial multiplication is thought to be due to the inhibition of the phagocytic cells of the insect by the fungus-produced toxin; this assumption is based on the observations of the effect of penicillin on human leucocytes (Florey and Jennings 1942). Tests to show the effect of the fungus toxin on bacteria were made. *Escherichia coli* (Migula) Castellani and Chalmers. *Staphylococcus aureus* Rosenbach, *Serratia marcesens* Bizio, and *Bacillus subtilis* (Ehrenberg) Cohn were tested. Inhibition of bacterial growth resulted only with *Serratia marcesens,* a confirmation of the finding of Masera (1934). These tests were made by the agar disc and strip methods of Wilkins and Harris (1944, a, b).

Paralysis did not occur within two hours of exposure to germinating spores in tests with the following insects: tussock moth larvæ (*Hemerocampa vetusta* Bdv.), Eastern tent caterpillar larvæ (*Malacosoma americana* Fabr.), German roach adults (*Blattella germanica* L.), Southern armyworm larvæ (*Prodenia eridania* Cram.), grasshoppers (Locustidæ), and housefly maggots (*Musca domestica* L.). All these forms are susceptible to the

parasitic action of *B. bassiana* with paralysis occurring as the first stage of attack. It is thought that these negative results can be attributed to the small amount of insect body surface in contact with the toxin-bearing filter paper in proportion to the volume of the insect. This does not hold true for the housefly maggots; this form is extremely resistant to even parasitism by *B. bassiana.*

A solution of penicillin, 2500 units per ml. of water, was used as a medium for mosquito larvæ, *Anopheles quadrimaculatus* Say. This treatment resulted in mortality of 14 of the 17 test insects; there was no death in the checks. Exposure was for 24 hours. A dust made up of 95,000 units of penicillin in two grams of wheat flour dusted on Petri dishes as described in Table I, produced no kill of housefly adults in 24 hour exposures.

In these tests 9.0 cm. Petri dishes were used. Almost one-eighth gram of spore dust was put on each wet filter paper. From these tests it is concluded that germination takes place only in the presence of a droplet of water, 90 percent relative humidity is not sufficient.

The variations in the quantitative results reported here can be attributed to a few causes. These include: experimental error; insufficient replication; variations in the virulence of the spores, they were not of a monosporous culture nor at the time of testing, of the same age; variations in the ability of the insects to withstand the experimental conditions, note the variations in check mortalities.

A possible explanation of why the bacterial type death in fungus attacked insects has not heretofore been mentioned in the literature, includes variation in method of experimentation and in size of test insects. In these tests the spores were permitted to germinate on a filter paper before exposure of the insect, in contrast to most other tests where the insect was dusted and germination was sporadic or limited. Also, early bacterial type death was noted only in tests with small insects, in contrast to many of the previously reported tests using corn borer larvæ and other large insects.

TABLE I

Tests were made of the effect of *Beauvaria bassiana* on housefly adults in laboratory tests. The procedure for each test is listed. Alive refers to those able to walk; Dead includes those paralyzed, or unable to walk.

PROCEDURE		Alive	Dead
About 200 chilled houseflies were liberated in a moist chamber with a wet filter paper on the bottom. The chamber was dusted with 1.0 g. of 0.5 percent *B. bassiana* spores in wheat flour. The count was made after a three hours exposure.	Ck	95%	5%
	T	5%	95%
The above test was repeated in a moist chamber with 70 to 80 percent relative humidity with no wet filter paper in the chamber. The count was made after a 24 hour exposure.	Ck	95%	5%
	T	95%	5%
Ten flies were liberated in a moist chamber with a wet filter paper on the bottom and the humidity at condensation point. One percent *B. bassiana* spores had settled and germinated one half hour, before the flies were put in. Counts were made one hour and 21 hours after the flies were put in.		1 hour	
	Ck	10	0
	T	10	0
		21 hours	
	Ck	10	0
	T	0	10
This test was similar to those listed above. The check chamber was washed out with $CuSO_4$ solution, five percent, before the flies were put in. The flies were in flying condition, not previously chilled as in the above tests. The spores were allowed one hour to germinate before the flies were put in. Counts were made at the end of two and 24 hours of exposure.		2 hours	
	Ck	7	0
	T	3	4
		24 hours	
	Ck	7	0
	T	0	7
In all tests after this, Petri dishes were substituted for a moist chamber. Wet filter paper was placed on the bottom of each dish. The spores were allowed one hour to germinate before the flies were put in. Ten flies were put into each dish. The count was made at the end of three hours of exposure.	Ck	64	16
	T	11	69
This was a test of newly prepared two percent *B. bassiana* spore dust. The spores were produced from a new culture obtained from the American Type Culture Collection, Washington,	Ck–1	40	12
	–2	36	12
	T–1	0	36

TABLE I (CONTINUED)

PROCEDURE		Alive	Dead
D. C. The test was made as described before except many chilled flies were placed in each dish. Counts were made at the end of a 12 hour exposure period.	−2	0	36
This test contrasts the effectiveness of old spore dust (18 months) and new dust. The test was made in Petri dishes; ten flies were put in each dish. The counts were made at the end of a 12 hour exposure period.		Old spores	
	Ck	14	6
	T	3	17
		New spores	
	Ck	16	14
	T	3	27
The effect of ingestion of spores which had not germinated was investigated in this test. Flies were separately mounted on paraffin blocks and fed individually. The flies were fed at 12 hour intervals after a 24 hour conditioning period. The flies were fed three times each during the test. The count was made 24 hours after the last feeding.	Ck	7	3
	T	9	1
This test was the same as the above test. The duration of the test was five feedings. After 48 hours on the block, sucrose was added to the water containing spores which had not germinated. The count was made 24 hours after the last feeding.	Ck	6	4
	T	6	4
About 250 flies in insectary type cages were fed milk which had been dusted with 0.25 g. one percent *B. bassiana* spores. At the end of 24 hours milk was added to the dusted feeding dish. Counts were made at 24 and 48 hours after the initial dusting and feeding. The check cage was kept in another room in the building.		24 hours	
	Ck	98%	2%
	T	63%	37%
		48 hours	
	Ck	95%	5%
	T	0%	100%
This test was similar to the above test except the feeding dish had a much lighter inoculation of *B. bassiana* spores; it was air contaminated in the laboratory. At the end of 48 hours mycelia, later identified as *B. bassiana*, were seen in the milk dish. Counts were made at the end of 24 and 48 hours. Check cage was same as that listed above.		24 hours	
	Ck	98%	2%
	T	89%	11%
		48 hours	
	Ck	95%	5%
	T	0%	100%

TABLE I (Continued)

PROCEDURE		Alive	Dead
Petri dishes were prepared with wet filter papers and with spores which had settled and germinated. Twelve or 20 flies were placed in each dish. A thread ligatured the cervix of the flies preventing feeding. The count was made at the end of a 48 hour exposure.	Ck	24	8
	T	0	32
This test shows the effect of the fungus is not solely due to parasitic action. Petri dishes were prepared with a wet filter paper and the spores were allowed 12 hours to germinate. The plates were then autoclaved at 20 lbs. for 30 minutes. When the plates had cooled, ten flies were placed in each dish. Counts were made at the end of a 48 hour exposure.	Ck	9	1
		Old spores	
	T	5	5
		New spores	
	T	5	5

TABLE II

Tests were made of the effect of *Beauvaria bassiana* on potato tuber worm larvæ. All tests listed were in Petri dishes prepared with a wet filter paper on the bottom. The procedure for each test is described. Alive refers to alive, able at least to move; Dead includes those dead or paralyzed, all unable to move.

PROCEDURE		Alive	Dead
Petri dishes were prepared with one percent *B. bassiana* spores. The spores were allowed at least one half hour to germinate before the larvæ were put in. After a two hours exposure, the larvæ were dipped in a one percent $CuSO_4$ solution. The count was made 22 hours after dipping. There were about 20 larvæ in each dish. No fungus growth was noted on the dead larvæ, one week after dipping.	Ck	64	14
	T	22	56
The above test was repeated with only one hour exposure of the larvæ to the spores. The count was made at the end of the one hour exposure; no $CuSO_4$ dip was included. Each dish contained 15 larvæ.	Ck	13	2
	T	6	9

TABLE II (CONTINUED)

PROCEDURE		Alive	Dead
Petri dishes were prepared as above. The spores were allowed a two hour germination period; the dish was then flooded with $CuSO_4$ solution. Larvæ were placed on the plate and counted at the end of a 22 hour exposure. There were 18 larvæ in each dish.	Ck	13	5
	T	0	18
Petri dishes were prepared in the usual manner. Ligatures were tied in the prothoracic region of the larvæ. A one and one half hour exposure of the larvæ was followed by a $CuSO_4$ dip. The count was made at the end of the 24 hour period after dipping.	Ck	5	0
	T	3	2
Petri dishes were prepared as above. The larvæ were ligatured in the prothoracic region and were exposed for 24 hours. The count was made at the end of the 24 hour exposure period.	Ck	12	2
	T	3	11
Petri dishes were prepared as above; the spores were allowed 12 hours to germinate. The plates were then autoclaved at 15 lbs. for 20 minutes. The larvæ were left on the plate 72 hours before counting.	Ck	8	2
	T	2	8
The preceding test was repeated with the exposure period of the larvæ reduced to 48 hours before the count was made. Autoclaved plates were compared with non-autoclaved. Twenty larvæ were in each Petri dish. Checks 1 and 2 are No Flour and Plus Flour respectively; Checks 1 and 2 and Test 1 were not autoclaved. Check 3 was Plus Flour; Check 3 and Test 2 were autoclaved (15 lbs., 20 min.).	Ck–1	6	14
	–2	8	12
	–3	6	14
	T–1	0	20
	–2	1	39
The above test was repeated with a 22 hour exposure of the larvæ to the autoclaved plate. Checks 1 and 2 were Plus Flour; Check 1 and Test 1 were not autoclaved. Check 2 and Test 2 (both three plates each) were autoclaved. The high mortality in Check 1 was thought to be due to an excess of water in the plate.	Ck–1	6	14
	–2	44	16
	T–1	0	20
	–2	18	42

TABLE III

Tests were made of the effect of *Beauveria bassiana* on dock beetle adults in Petri dishes in the laboratory The procedure for each test is described. Alive refers to alive, able at least to move; Dead includes dead or paralyzed, all unable to move.

PROCEDURE		Alive	Dead
Petri dishes were prepared with wet filter papers		2 hours	
and dusted with one percent *B. bassiana* spores.	Ck	10	0
The spores were allowed at least one half hour	T	0	10
to germinate before the beetles were put in.			
Ten dock beetle adults, equal numbers of males		22 hours	
and females, were placed in each dish. The	Ck	10	0
first count was made at the end of two hours;	T	0	10
the beetles were dipped in $CuSO_4$ and counted			
again 22 hours later. At the end of 24 hours			
all Test insects were dead; at the previous count			
some were only paralyzed.			
This test included a twelve hour exposure of the	Ck	6	4
beetles to the spores. There was no $CuSO_4$	T	1	9
treatment. The count was made at the end of			
the exposure period.			
The above test was repeated with the new spore	Ck	6	4
dust. The previous test had been made with	T	1	9
dust about one year old.			
Petri dishes were prepared in the usual man-	Ck	4	1
ner. The spores were allowed a 12 hour ger-	T	2	3
mination period. The plates were then auto-			
claved at 20 lbs. for 30 minutes. The beetles			
were exposed for 48 hours before the count was			
made.			

CONCLUSIONS

The germinating spores of *Beauveria bassiana* produce a toxin which is a contact poison. This toxin may cause paralysis of the insect even in advance of hyphal invasion. In addition, it is concluded that the poison is toxic to the phagocytic cells of the insect, since, in laboratory tests histolysis by intestinal bacteria usually follows paralysis. The toxin has the same effect when ingested.

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